DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

NOTES ON THE CULTURING OF MICROSCOPIC ORGANISMS FOR THE ZOÖLOGICAL LABORATORY

General.—The culture of micro-organisms, especially the Protozoa, is an old topic among biologists. Leeuwenhoek in his quaint language records not only the finding of Protozoa in various natural waters but also in infusions of pepper. He was thus the first to make cultures of the Protozoa. Leeuwenhoek's cultures were largely due to a series of accidents or to haphazard experiments. Many zoölogists of today use this haphazard method of making cultures. It is the usual practice to collect a mass of vegetable material from a pond, stream or other body of water. This mass is placed in almost any kind of a dish, and left in strong light or in darkness, covered or uncovered as long as the preparator can stand the odor. From these haphazard methods he gets some surprising results but quite frequently not at the time when wanted. If he has made these cultures with some particular protozoan in mind, he is quite likely to find the species mixed (if he finds it at all) with several other species. When through using these cultures or when they are spent he throws them out and makes more in the same haphazard fashion.

These have been and are yet to a large extent the methods of culturing Protozoa as practiced by the zoölogist. Bacteriologists, however, have developed very careful methods of culturing bacteria and some few species of Protozoa. They make use of media containing known and measured ingredients, kept under controlled conditions. The response of the organisms to particular media and to particular conditions is characteristic and the habit of growth

is an aid in diagnosis of the species under observation. The bacteriologist cannot use haphazard methods and secure results. Botanists also are making use of exact methods in the culturing of plant organisms as algæ, fungi and even higher green plants. The use of exact methods by botanists has been promoted by the fact that many of them are trained in bacteriological methods and in the exact methods of plant physiology. Their research in the life histories and in the metabolism of lower plants has demanded the use of exact methods. The zoölogist, too, must come to the use of quantitative methods in the culturing of many of the lower forms of laboratory animals. He is, however, more or less dependent on the bacteriologist and the botanist to show him the methods of rearing the food organisms for his Protozoa or his worms. The zoölogist can, indeed, help himself very greatly if he will learn to use the cultural methods of the bacteriologist and the botanist. It is not enough that some zoölogists make use of these methods but having worked out methods that are successful they should by early publication make their methods available to others. The writer has no sympathy with the disinclination of some workers to make their methods known. There is a real need among amateur zoölogists, teachers and investigators for a greater knowledge of technical methods and such information ought to be made readily available.

Some criticisms of the old methods may be given and some suggestions offered concerning food supply, dishes, light, inoculation and culture media.

Food Supply.—One of the great faults of the old methods of culturing micro-organisms was in the failure to maintain a stock of food materials in cultures that had begun to yield. When the culture was made it was heaped high with food materials which were never replaced as they became exhausted by the action of bacteria. With depletion of the organic matter the crop of bacteria or of unicellular algæ which furnished pasturage for many of the Protozoa rapidly diminished in numbers and the Protozoa decreased to the vanishing point. For this reason it has been the custom to bring in cultures every few days in the hopes of having one culture ready when wanted. It should be borne in mind that if the number of animals in a culture is to be maintained their food supply must be replen-

ished. This can be done by adding hay, manure from various sources or manure solution, or other vegetable or organic material which does not quickly become acid in the process of decay. Rapid souring of culture medium is fatal to most Protozoa. The writer has never been very successful in growing Protozoa in media containing much of such carbohydrates as occur in wheat.

Culture Dishes.—Even the kind of a dish in which the culture is maintained is of importance. The best dishes are shallow and have a diameter considerably greater than their depth. Covered glass dishes ca 200 mm. in diameter by 80 mm. in depth and known as bacteria dishes or culture dishes are excellent but not indispensable. The writer has used Stender dishes 50 mm. in diameter by 30 mm. in depth with success although a small culture is more difficult to maintain than a larger one. Fruit jars, battery jars and even aquarium jars are satisfactory if the liquid of the culture is not too deep. Earthenware jars and crocks are satisfactory for forms preferring the dark. Keep all cultures covered.

Light.—As to light, diffuse light or even darkness may be used for most forms except those which feed on a green or blue-green plant or which like Euglena need light for photosynthesis. Even in these cases the diffuse light and more even temperature of a north window are to be preferred to direct sunlight and the accompanying violent fluctuations of temperature.

Inoculation.—If in the wild cultures the desired organisms appear in numbers new cultures may be inoculated with a small quantity of material from the old culture, taking precaution to add the food organism also and to avoid contamination with other organisms. Thus one may become reasonably free from the necessity of frequent trips to pond or stream to collect vegetation for cultures. The culturist should study the food habits of the organism and strive in preparing the new culture to provide the proper medium for the development of the food organism and then he must provide proper conditions of light, temperature and gaseous exchange. If by observation or experiment he can determine the ingredients to be used in his culture and the conditions for the growth of the food organisms he should be able to maintain cultures almost indefinitely.

Culture Media.—Various culture media have been suggested for general and special Protozoa cultures. The writer has confined his efforts to a few media. One of the most successful of these for general cultures is made of sterile hay and filtered tap-water. To prepare this medium timothy hay is made into small compact bundles which are tightly wrapped in several layers of cheese cloth. The bundles are sterilized once in a steam autoclave at 15 to 17 lbs. pressure for 15 minutes or more, then dried over a steam radiator or in a drying oven. This sterilization is not intended to kill bacteria or spores of fungi but to kill encysted Protozoa if any be present. The tap-water is filtered through a paper filter, preferably into sterile dishes. The idea here is to free the water of any Protozoa which are always present in tap-water of this city but not necessarily to get rid of the bacteria. Ten grams of the sterile hay to two liters of filtered tap-water makes a very satisfactory medium for many kinds of Protozoa. These proportions, however, can be varied considerably. When a culture has been started in this medium by inoculation from a previous culture it may be maintained by the addition of one or two grams of sterile hay per week and as much filtered water as is necessary to maintain the original level of liquid.

Boiled hay solution has been tried repeatedly and has never given good results, apparently because of the development of toxic substances. It usually soon acquires an odor somewhat resembling butyric acid and when in this condition if Paramecia are placed in it they die in a very few minutes.

Manure solution made by boiling fresh horse or cow manure or human feces in tap-water and diluting with filtered tap-water has given excellent results in culturing Euglena and some unicellular green algæ as Scenedesmus. Any of these may serve as food organisms for some of the Protozoa. Unfortunately the proportions in which the manure solutions were made have not been kept. It has, however, been the experience of the writer that the proportions may be widely varied. Manure solutions may be added to the hay cultures with beneficial results.

The writer has found the filtered tap-water superior to rain-water which is lacking in many of the salts necessary for most organisms. Distilled water has never been used because it has been

simpler to use the filtered tap-water than to add salts in the proper proportions to the distilled water. Boiled tap-water is inferior to filtered tap-water probably because boiling precipitates a large proportion of the salts and drives off the oxygen and carbon dioxide. In the event that the tap-water has been treated with chlorine it may be necessary to use filtered pond or stream water or spring water.

Culture of Amœba.—Two of the writer's students, Miss Knisely and Mr. Welch, have worked out methods of culturing ameba on solid media. The results of this work were published in in these Transactions, vol. 34, pp. 21-25.

The writer's work in culturing ameba has been incidental to the securing of materials for class use and some of the results attained have been more or less accidental. Ameba cultures have been secured in many ways of which only a few are related.

During the summer of 1916 small amebæ, probably soil amebæ, occurred in great abundance in a culture made from unwashed radishes pulled from a damp garden. No efforts were made to maintain the culture and the amebæ soon disappeared.

On Aug. 29, 1916, some lily-pads and pond water were taken from First Sister Lake, near Ann Arbor. This culture yielded among other Protozoa a fair number of large amebæ. On Dec. 29, 1916, a sterile-hay-filtered-tap-water culture was made up in a bacteria dish and some scum from the preceding culture was added. In this culture a slender diatom and the ameba established themselves and have thriven to date. This ameba is a large rounded sluggish form measuring nearly 100 microns in extreme dimension. Its surface is wrinkled. It puts forth few pseudopodia, has a thick hyaline ectosarc, finely granular entosarc, large contractile vacuole, and a well-defined ellipsoidal nucleus within a wider clear area. Specimens have usually been very numerous in this culture and since there have been very few other organisms present it has been excellent for student use. To this culture were added at intervals of one to several weeks a few grams of sterile hay until June 21, 1917. At this time the culture was in good condition. The diatoms were very numerous on the bottom of the dish where the sluggish amebæ were abundant. There were also many more active amebæ

resembling *A. proteus*. From June 21 until Aug. 30 no hay was added to this culture and it was not examined meanwhile. On the latter date amebæ of the above mentioned kinds were found but not abundantly. About 2 grams of hay were added Aug. 30 and on Sept. 19 both kinds of amebæ were quite abundant.

On Jan. 9, 1917, a subculture in a sterile-hay-tap-water medium was made from the culture above mentioned. For a long time it yielded amebæ altho less abundantly than the parent colony, but on June 21 it was producing quantities of diatoms upon which large numbers of the sluggish ameba were feeding. This culture had more hay than the parent culture. It was uncared for from June 21 until Aug. 30 when a few amebæ were found. Hay was added Aug. 30 and on Sept. 19 the amebæ were more abundant.

The above cultures have always been kept near a north window in diffuse light and have always been kept covered with a glass plate. Recent comparison of these amebæ with amebæ secured on Sept. 12, 1917, from the original source of these cultures shows that they have not changed in form or size.

Culture of Euglena.—Euglena occurs most abundantly in foul waters and is well known as an inhabitant of sewage-polluted waters. Euglena cultures have been maintained in luxuriance with almost no attention on the sterile-hay-filtered-tap-water medium for more than a year in the diffuse light of a north window. They also have been transplanted into manure solutions where they have produced a dense green scum over the surface of the water and down the sides of the jar and as far above the water on the sides of the jar as the film of moisture extended. After a time large numbers of these Euglenæ become encysted. It has been found by Dr. A. F. Shull of our laboratory that such Euglenæ soon emerge from the cyst if placed in a fresh manure solution. Thus the providing of Euglena for class use which formerly was the bugbear of the general zoölogy course is now a very simple matter.

Culture of Tetrastemma.—A fresh water nemertean worm which resembles Tetrastemma and has provisionally been assigned to that genus has appeared in cultures taken from First Sister Lake, near Ann Arbor, on July 12, 1916. Until September, 1916, this culture was maintained in a covered pint fruit jar, then was put in

a bacteria dish with filtered tap-water and some sterile hay. Tetrastemma was first noted in November. The culture was maintained by adding a few grams of sterile hay at intervals of one or more weeks. During most of the winter this organism was abundant. On March 23, 1917, this worm was present in large numbers, but by June 21 its numbers were greatly reduced. On Aug. 30 a single specimen was found in a subculture.

Culture of Pristina.—In the culture in which Tetrastemma appeared and at the same time the oligochæte worm, *Pristina sp.* appeared and was maintained in considerable abundance until late February and although on March 23 it was on the decline it was still present in cultures on June 21. Subcultures were made from time to time during the winter in which this worm thrived. The species has disappeared in these cultures during the summer. The cultures of Tetrastemma and of Pristina were maintained just as Paramecium cultures were maintained. In fact during the greater part of the time Paramecia were very numerous in all the cultures of these worms.

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EFFECTS OF THYROID ON PARAMECIUM

Shumway (J. Exp. Zool., Apr., 1917) cites a number of interesting results from culturing Paramecium in emulsions and suspensions of thyroid. Among these are,—that the division rate is increased 65% over the controls; that this acceleration of rate is greatest at the time when the controls themselves are dividing most rapidly, and hence the curves show the same general rhythms as those of the controls; that the number of contractile vacuoles increases from two to three, indicating a disturbance of the excretory function; that large vocuolation takes place in the protoplasm, which is believed to have an excretory significance, altho the vacuoles do not pulsate. They were often observed to burst, freeing their contents internally.